Journal of Assisted Reproduction and Genetics, Vol. 19, No. 7, July 2002 (© 2002)

Clinical Assisted Reproduction

Comparison Between a Single Dose of Goserelin (Depot) and Multiple Daily Doses of Leuprolide Acetate for Pituitary Suppression in IVF Treatment: A Clinical Endocrinological Study of the Ovarian Response

Selmo Geber,[1,2,3] Liana Sales,[1] and Marcos A. C. Sampaio[1]

Submitted November 19, 2001; accepted February 18, 2002

***Purpose*:** Compare the efficacy and safety of two different GnRHa, used for pituitary suppression in IVF cycles.
***Methods*:** A total of 292 patients using depot goserelin (Group 1) and 167 using daily leuprolide acetate (Group 2) were compared. Days required to achieve pituitary function suppression, duration of ovarian stimulation, total dose of HMG, number of aspirated follicles, number of oocytes retrieved, and presence of functional ovarian cyst were analyzed.
***Results*:** The time taken to achieve downregulation was similar. The mean number of ampoules used for superovulation was higher in Group 1; however, this difference was observed only for patients >40 years old that started GnRHa in the follicular phase. There was no difference between the two groups in the duration of superovulation, in the number of follicles aspirated, and the number of oocytes retrieved. In the group of patients with >40 years the incidence of ovarian cysts was higher in Group 2.
***Conclusions*:** Both routes of GnRHa have similar effects for pituitary suppression and ovulation induction in assisted reproductive technology. Therefore the long-acting GnRHa is an excellent option, as only a single subcutaneous dose is necessary, decreasing the risk of the patient to forget its use and, most important, it does not interfere in the patient's quality of life.

KEY WORDS: Downregulation; functional cysts; GnRHa; IVF; ovarian response.

INTRODUCTION

One of the most important advances in the field of assisted reproductive technologies was the development of the gonadotrophin-releasing hormone analogues (GnRHa) (1). The GnRHa are used in combination with menotropins or recombinant FSH (follicle-stimulating hormone) to induce folliculogenesis for controlled ovarian hyperstimulation in women undergoing in vitro fertilization (IVF). The benefits from the use of the GnRHa in assisted reproductive methods derive principally from the absence or reduction in the incidence of premature luteinizing hormone (LH) surge and premature luteinization (2–4). Therefore, the incidence of cycle cancellation has decreased, and the incidence of embryo transfer per cycle started has increased (5,6). The possibility to avoid LH surge also improved patient and physician convenience, allowing oocyte retrieval to be performed only on week days (7). Moreover, lower concentrations of bioactive LH also decrease local ovarian androgen concentrations, enhancing estrogen/androgen ratio and improving the oocyte performance (8).

[1] ORIGEN, Centro de Medicina Reprodutiva, Av Contorno 7747, Belo Horizonte, Minas Gerais, CEP 30110120, Brazil.
[2] Department of Obstetrics and Gynaecology, Federal University of Minas Gerais, Minas Gerais, Brazil.
[3] To whom correspondence should be addressed; e-mail: sjgeber@bhnet.com.br.

1058-0468/02/0700-0313/0 © 2002 Plenum Publishing Corporation

The other positive effects of the GnRHa are the increasing in the number and synchronization of developing follicles (9,10), increasing in the average number of oocytes obtained at retrieval (11), augmentation of oocyte maturation synchrony, and most important, its use has raised pregnancy rates in IVF treatment when compared with cycles not using GnRHa (12).

Many protocols that combine GnRHa and gonadotrophins have been proposed within the last years; however, the long protocol have been the most widely used because of its better results (13,14). The long protocol aims to obtain pituitary suppression before ovulation induction. The GnRHa is started either in the early follicular phase or in the midluteal phase of the previous cycle and must be continued at least until the induction of oocyte maturation. The other advantage of this regimen is the possibility to schedule patients for treatment.

Several GnRHa with different chemical structure and formulation, biological potencies, serum half-life, dosing requirement, and various routes of administration have been developed. The selection of one specific agent for use in IVF treatment depends mostly on its benefits for the treatment, i.e., good results in obtaining downregulation, high number of developing follicles and oocytes retrieved. It is also important that the chosen GnRHa does not increase the length and the costs of treatment. Moreover, the clinician experience with the analogue and the impact of the route of administration on the patients' quality of life are also fundamental to obtain good treatment results.

Although many studies have been performed to find out whether one compound was better than the others, no data suggested clinical superiority of any GnRHa (15,16). The aim of our prospective randomized study was to compare the efficacy and safety of two different GnRHa with different route of administration, used for pituitary suppression in IVF cycles using the same protocol.

MATERIAL AND METHODS

A total of 459 patients referred for IVF treatment at the ORIGEN, Centro de Medicina Reprodutiva, were included in this prospective study, performed between October 1995 and March 1998. Patients were randomized to receive either one dose of subcutaneous goserelin (Zoladex, Zeneca, Brazil) or subcutaneous daily leuprolide acetate (Lupron, Abbot, Brazil), according to the phase of the year they started treatment, i.e., starting first semester used goserelin and 2nd semester used leuprolide. All patients that used the appropriate GnRHa were included in the study. The decision of starting on the follicular or luteal phase was made by the patients according to their own comfort. This study was approved by our institutional ethics committee according to the code of ethics of the Conselho Federal de Medicina (Brazilian National Medical Council).

Patients

A total of 292 patients (63.6%) used goserelin (Group 1), and 167 (36.4%) used leuprolide acetate (Group 2). All patients had long protocol for pituitary suppression and the same stimulation protocol for ovulation induction regardless of the type of GnRHa used. The groups were comparable in indications for IVF and age ($p > 0.05$). Indications for IVF were male factor, tubal disease, endometriosis, and unexplained infertility.

Stimulation Protocol

Treatment started on Day 2 (follicular phase) or Day 21 (luteal phase) of the menstrual cycle with GnRHa. Patients of Group 1 received subcutaneous administration of 3.6 mg of goserelin (Zoladex, Zeneca, Brazil) and of Group 2 started daily subcutaneous administration of 1 mg of leuprolide acetate (Lupron, Abbot, Brazil) for suppression of the pituitary function. To confirm pituitary suppression, serum estradiol (E_2) levels and vaginal ultrasound were performed 7–10 days later (according to patient's convenience). If the E_2 concentration was less than 30 pg/mL and the ultrasound showed an endometrial thickness of less than 3 mm, patients were considered ready to start ovulation induction. If patients were not ready, serum E_2 and vaginal ultrasound were repeated every other day until suppression was achieved.

After confirmation of suppression, all patients were superovulated with daily human menopausal gonadotrophin (HMG; 75 IU of FSH/LH per ampoule) intramuscular injections. The starting dose of HMG was defined according to patient's age, and the dose was tailored according to the E_2 levels and follicular growth monitored by vaginal ultrasound (Tosbee, Toshiba, Japan). Human chorionic gonadotrophin (hCG; 10000 IU) was given when at least two follicles reached a mean size of 17 mm with concordant estradiol levels. Oocyte retrieval was performed ~34 h after hCG injection by vaginal ultrasound

guided aspiration. IVF or intracytoplasmic sperm injection procedure were performed as previously described (17).

Cysts Aspiration

The presence of a functional ovarian cyst was considered when observed at ultrasound and associated with elevated E_2 levels when patients were examined to confirm downregulation. If pituitary function suppression was not achieved within 15 days, ovarian cysts were aspirated by vaginal ultrasound guided suction.

Statistical Analysis

Statistical analysis to compare the two groups was performed using the Kruskal–Wallis test for the following criteria evaluated: days required to achieve pituitary function suppression, duration of ovarian stimulation, total dose of HMG (number of ampoules), number of aspirated follicles, number of oocytes retrieved. To compare the presence of functional ovarian cyst, age of patients and menstrual cycle phase to start GnRHa, between the two groups, chi-square test and Fisher's exact test were performed. A difference was considered significant when $p < 0.05$.

RESULTS

The mean ± SD age in Group 1 was 34.4 ± 4.7 (range 23–44 years) and 34.3 ± 5.4 (range 20–44 years) in Group 2 (not significantly different). Downregulation was achieved in all patients in both groups before superovulation. In Group 1, 78.3% of the patients started GnRHa on the follicular phase, and in Group 2, 80.6% (not significant). All cycles included in the study reached the criteria for oocyte retrieval.

In both groups, the time taken to achieve downregulation was similar: 14.2 ± 5.5 days for Group 1 (range 8–60) and 13.8 ± 6.3 days for Group 2 (range 7–35). However for the patients that started treatment in the follicular phase and for the patients with ≤30 years, the time was statistically significantly longer in Group 1 (Table I).

The mean number of ampoules used for superovulation was statistically higher in Group 1 (range 12–118 in Group 1 and 13–91 in Group 2). However, this difference was observed only for patients that started GnRHa in the follicular phase, and in the group of patients >40 years old (Table II).

Table I. Time Taken for Downregulation to be Achieved After Administration of Goserelin or Leuprolide Acetate According to Menstrual Cycle Phase of Starting Treatment and Age

	Goserelin		Leuprolide acetate		
	n	(days)	*N*	(days)	*p*
Follicular phase	227	14.2 ± 6.1	133	13.4 ± 6.4	*0.011*
Luteal phase	65	14.2 ± 2.9	34	15.2 ± 5.7	0.66
≤30 years	63	13.0 ± 4.0	35	10.6 ± 4.2	*0.001*
31–35 years	108	14.5 ± 5.0	59	14.3 ± 6.1	0.64
36–40 years	94	14.5 ± 7.1	55	13.5 ± 5.8	0.20
>40 years	27	14.7 ± 4.1	18	18.7 ± 8.6	0.19
Total	292	14.2 ± 5.5	167	13.8 ± 6.3	0.054

Kruskal–Wallis test.
Days: mean ± SD.

The mean ± SD number of days of HMG taken to reach criteria for hCG administration was 11.9 ± 5.3 days in Group 1 (range 8–18) and 11.6 ± 3.4 days in Group 2 (range 7–17). There was no difference between the two groups if the patients started GnRHa in the follicular or luteal phase. The duration of superovulation was also not statistically different between the two groups, when considered by age groups (Table III).

There was no significant difference between the two groups in the number of follicles aspirated and the number of oocytes retrieved. In the goserelin group, a mean number of 11.2 follicles were punctured (range 2–33), and 12.8 oocytes were retrieved (range 0–52). In the leuprolide acetate group, a mean number of 13.6 follicles were punctured (range 2–76) and 12.4 oocytes were retrieved (range 0–78). These numbers were also not different whether the patients started GnRHa in the follicular or luteal phase or according to age group (Table III).

The presence of ovarian cysts was observed in 56 patients in Group 1 (19.2%) and in 39 patients in Group 2

Table II. Number of Ampoules of HMG Used for Superovulation After Administration of Goserelin or Leuprolide Acetate According to Menstrual Cycle Phase of Starting Treatment and Age

	Goserelin		Leuprolide acetate		
	n	(ampoules)	*N*	(ampoules)	*p*
Follicular phase	227	47.6 ± 15.6	133	44.4 ± 14.5	*0.037*
Luteal phase	65	45.8 ± 14.4	34	43.4 ± 15.2	0.511
≤30 years	63	36.2 ± 11.7	35	32.9 ± 6.5	0.366
31–35 years	108	42.5 ± 10.1	59	41.4 ± 12.1	0.346
36–40 years	94	52.9 ± 12.5	55	49.4 ± 14.7	0.117
>40 years	27	70.6 ± 16.1	18	59.1 ± 14.7	*0.017*
Total	292	47.1 ± 15.4	167	44.1 ± 14.6	*0.038*

Kruskal–Wallis test.
Ampoules: mean ± SD.

Table III. Duration of Superovulation, Number of Follicles Aspirated, and Number of Oocytes Retrieved in Patients Downregulated by Goserelin or Leuprolide Acetate According to Menstrual Cycle Phase of Starting Treatment and Age

	Goserelin			Leuprolide acetate		
	Days of HMG	Follicles	Oocytes	Days of HMG	Follicles	Oocytes
Follicular phase	11.9 ± 6.0	11.2 ± 6.3	12.6 ± 7.7	11.4 ± 1.7	13.4 ± 10.1	12.5 ± 10.1
Luteal phase	11.7 ± 1.8	11.0 ± 6.7	12.9 ± 9.3	12.8 ± 6.9	14.1 ± 9.1	11.3 ± 7.8
≤30 years	11.2 ± 1.7	15.6 ± 7.0	18.1 ± 9.3	11.0 ± 1.8	19.3 ± 8.8	17.7 ± 8.9
31–35 years	11.6 ± 1.8	11.7 ± 5.9	13.5 ± 7.4	11.4 ± 1.5	13.7 ± 8.5	12.5 ± 8.0
36–40 years	12.5 ± 9.0	9.2 ± 4.8	10.1 ± 6.3	12.3 ± 5.4	12.6 ± 14.4	11.2 ± 11.1
>40 years	12.4 ± 1.8	5.9 ± 5.3	6.7 ± 6.2	11.9 ± 2.0	5.6 ± 3.6	5.1 ± 3.3
Total	11.9 ± 5.3	11.2 ± 6.4	12.8 ± 8.2	11.6 ± 3.4	13.6 ± 9.9	12.4 ± 9.6

Note. Days of HMG, number of follicles aspirated, and number of oocytes retrieved are mean ± SD.
Kruskal–Wallis test.

(23.5%). The difference was not significant between the two groups. There was also no difference between the two groups if the patients started GnRHa in the follicular or luteal phase. However in the group of patients with >40 years, the incidence of ovarian cysts was statistically higher in Group 2 (Table IV). All ovarian functional cysts were aspirated, and no complications were recorded.

DISCUSSION

Our study evaluates two different GnRHa with different formulation and routes of administration. Some studies have previously demonstrated the efficacy of one dose of subcutaneous goserelin (15,16,18,19) for pituitary suppression, before ovarian hyperstimulation in women undergoing IVF; however, only a small number of patients have been evaluated in a prospective randomized study. Tapanainen *et al.* (15) evaluated 49 patients that underwent IVF, using goserelin for pituitary suppression, starting at the luteal phase for a long protocol treatment, that were compared to 51 women using buserelin. The only observed difference was a higher number of ampoules needed for follicular maturation in the goserelin group. To our knowledge this is the second prospective randomized study comparing one dose of goserelin to daily administration of GnRHa, the first comparing goserelin to leuprolide acetate and the study with the highest number of patients using goserelin.

Table IV. Incidence of Ovarian Cyst Formation After Administration of Goserelin or Leuprolide Acetate According to Menstrual Cycle Phase of Starting Treatment and Age

	Goserelin	Leuprolide acetate	*p*
Follicular phase	48 (21.2)	34 (25.8)	0.316
Luteal phase	8 (12.9)	4 (12.5)	1.000
≤30 years	10 (15.9)	2 (5.7)	0.203
31–35 years	22 (22.4)	14 (24.1)	0.574
36–40 years	21 (23.6)	15 (27.3)	0.520
>40 years	3 (11.1)	8 (44.4)	*0.016*
Total	56 (19.2)	39 (23.5)	0.282

Values are absolute numbers and percentages, with the latter in parenthesis.
chi-square test.

We did not include laboratory data, such as fertilization rates, embryo quality, number of transferred embryos, and pregnancy rates, in order to avoid any study bias. As during the study time we have used different culture media preparation, different criteria for intracytoplasmic sperm injection, and different stage for embryo transfer, we decided not to include these results as it might have biased the results. We do understand that pregnancy rates are the most important results in infertility treatment studies; however, the main objective of our study was to analyze the endocrine effects of both preparation in the ovaries.

The choice for the long protocol used for all patients was based on the results observed in the literature showing higher implantation and pregnancy rates (4,13). The starting day of the GnRHa was either Day 2 or Day 21 of the menstrual cycle, and downregulation was achieved in all patients before superovulation. Although it has been described that the long protocol initiated in the midluteal phase might be more effective and with a lower incidence of functional ovarian cysts formation (20), our results do not confirm such data. In fact, we observed that irrespective of the phase patients started GnRHa, the same time was needed to achieve downregulation, in both treatment groups. This lack of difference was also observed for functional cyst formation.

We observed that in the group of patients that used single dose preparation (goserelin), only women ≤30 years and that started at follicular phase needed more time to achieve downregulation. As all patients decided by themselves when to return after starting

GnRHa for confirmation of pituitary suppression (7–10 days later, according to patient's convenience), we can assume that those who were in use of daily administration of GnRHa decided to perform their first ultrasound and estradiol earlier (7th day) in order to minimize the use of injections. On the other hand, patients that were not using daily injections for down-regulation might have delayed the return to the last day (10th day). This can explain the fact that only this small and selected group of patients has shown this difference, and therefore we believe that this was a casual finding. The results observed by Oyesanya *et al.* (16) however have demonstrated that patients that used goserelin needed less time to reach down-regulation. Porcu *et al.* (21) did not find any difference in the time to reach desensitization when comparing depot (leuprorelin) and daily (buserelin) GnRHa.

Some authors (22) have described higher follicular recruitment and oocyte retrieval when GnRHa was commenced in the early follicular phase. These results are not in concordance with our data that showed similar number of follicles punctured and oocytes retrieved in both groups, irrespective of the cycle phase patients started GnRHa. Also, there were no differences in the time required for follicular development. These results are similar to those obtained by Tapanainem *et al.* (15) which compared goserelin to buserelin acetate, and demonstrate no differences in the clinical outcome. The same was observed by Oyesanya *et al.* (16) and Alvarez *et al.* (23).

Our results have demonstrated that patients in the goserelin group needed more ampoules of HMG. This result is similar to the findings observed by Tapanainen *et al.* (15); however, in our study this was observed only in a small group of patients, i.e., >40 years old that used goserelin starting in the follicular phase. Alvarez *et al.* (23) demonstrated that a long administration of GnRHa has no effect upon ovarian response in IVF cycles.

The presence of functional ovarian cysts was observed in 56 patients in Group 1 (19.2%) and in 39 patients in Group 2 (23.5%). Although the incidence was higher than that observed by Sampaio *et al.* (24), it was similar in both groups and did not interfere in the treatment as all cysts were drained and no complications were related to the procedure. We observed a higher incidence of functional ovarian cysts in the group of patients >40 years old using leuprolide acetate. This result is not in agreement with Tarlatzis *et al.* (25) that studying three groups of patients concluded that cyst formation does not seem to be related to the use of a specific GnRHa, its short- or long-acting form, or the mode of administration. Their study however was performed in a small number of patients (172) that might explain the difference.

In conclusion, our results demonstrate that both routes of GnRHa have similar effects for pituitary suppression and ovulation induction in assisted reproductive treatment. Therefore the long-acting GnRHa is an excellent option, as only a single subcutaneous dose is necessary, decreasing the risk of the patient to forget its use and therefore interfere with the down-regulation and, most important, it does not interfere in the patient's quality of life. For patients >40 years old it might be suggested another alternative to avoid the increased risk of cyst formation or of needing more ampoules.

REFERENCES

1. Fleming R, Adam AH, Barlow DH, Black WP, MacNaughton MC, Coutts JR: A new systematic treatment for infertile women with abnormal hormone profiles. Br J Obstet Gynaecol 1982;89:80–83
2. Neveu S, Hedon B, Bringer J, Chinchole JM, Arnal F, Humeau J, Cristol P, Viala JL: Ovarian stimulation by a combination of a gonadotropin-releasing hormone agonist and gonadotropins for in vitro fertilization. Fertil Steril 1987;47:639–643
3. Dor J, Shulman A, Pariente A, Levran D, Bider D, Menashe Y, Mashiach S: The effect of gonadotropin-releasing hormone agonist in the ovarian response and in vitro fertilization results in polycistic ovarian syndrome: A prospective study. Fertil Steril 1992;57:366–371
4. Smitz J, Van Den Abbeel E, Camus M, Devroy P, Touaye H, Van Steirtegheim AC: The effect of gonadotrophin-releasing hormone (GnRH) agonist in the follicular phase on in vitro fertilization outcome in normo-ovulatory women. Hum Reprod 1992;7:1098–1102
5. Tummon IS, Daniel SA, Kaplan BR, Nisker JA, Yuzpe AA: Randomized, prospective comparison of luteal leuprolide acetate and gonadotropins versus clomiphene citrate and gonadotropin in 408 first cycles of in vitro fertilization. Fertil Steril 1992;58:563–568
6. Hughes EG, Fedorkow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA: The routine use of gonadotrophin–releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: A meta-analysis of randomized controlled trials. Fertil Steril 1992;58:888–896
7. Dimitry ES, Bates SA, Oskarsson T, Margara RA, Winston RML: Programming in vitro fertilization for a 5- or 3-day week. Fertil Steril 1991;55:934–938
8. Polan ML, Daniele A, Russel JB, DeCherney AH: Ovulation induction with human menopausal gonadotropin compared to urinary follicle-stimulating hormone results in a significant shift in follicular fluid androgen levels without discernible differences in granulosa–luteal cell function. J Clin Endocrinol Metab 1986;63:1284–1291
9. Awadalla SG, Friedman CL, Chin NW, Dods W, Park JM, Kim MH: Follicular stimulation for in vitro fertilization using

pituitary suppression and human menopausal gonadotropins. Fertil Steril 1987;48:811–815
10. Porter RN, Smith W, Craft IL, Abduwahid NA, Jacobs HS: Induction of ovulation for in vitro fertilisation using buserelin and gonadotrophins. (Letter.) Lancet 1984;ii:1284–1285
11. Frydman R, Belaisch AJ, Parneix I, Forman R, Hazout A, Rainhorn JD, Fries N, Testart J: Comparison between flare up and down regulation effects of luteinizing hormone-releasing hormone agonist in an in vitro fertilization program. Fertil Steril 1988;50:471–475
12. Abdalla HI, Ahuja KK, Leonard T, Morris NN, Honour JW, Jacobs HS: Comparative trial of luteinizing hormone-releasing hormone analog/human menopausal gonadotropin and clomiphene citrate/human menopausal gonadotropin in an assisted conception program. Fertil Steril 1990;53:473–478
13. Tan SL, Kingsland C, Campbell S, Mills C, Bradfield J, Alexander N, Yovich J, Jacobs HS: The long protocol of administration of gonadotrophin-releasing hormone agonist is superior to the short protocol for ovarian stimulation for in vitro fertilization. Fertil Steril 1992;57:810–814
14. Filicori M, Flamigni C, Cognigni G, Falbo A, Arnone R, Capelli M, Pavani A, Mandini M, Calderoni P, Brondelli L: Different gonadotropin and leuprorelin ovulation induction regimens markedly affect follicular fluid hormone levels and folliculogenesis. Fertil Steril 1996;65:387–393
15. Tapanainen J, Hovatta O, Juntunen K, Martikainen H, Ratsula K, Tulppala M, Tuomivaara L: Subcutaneous goserelin versus intranasal buserelin for pituitary down-regulation in patients undergoing IVF: A randomized comparative study. Hum Reprod 1993;8:2052–2055
16. Oyesanya OA, Teo SK, Quah E, Abdurazak N, Lee FY, Cheng WC: Pituitary down-regulation prior to in-vitro fertilization and embryo transfer: A comparison between a single dose of Zoladex depot and multiple daily doses of suprefact. Hum Reprod 1995;10:1042–1044
17. Geber S, Sampaio M: Blastomere development after embryo biopsy: A new model to predict embryo development and to select for transfer. Hum Reprod 1999;14:782–786
18. Filicori M, Flamigni C, Cognigni G, Dellai P, Arnone R, Falbo A, Capelli M: Comparison of the suppressive capacity of different depot gonadotropin-releasing hormone analogs in women. J Clin Endocrinol Metab 1993;77:130–133
19. Dhont M, Onghena A, Coetsier T, De Sutter P: Prospective randomized study of clomiphene citrate and gonadotrophins versus goserelin and gonadotrophins for follicular stimulation in assisted reproduction. Hum Reprod 1995;10:791–796
20. Lockwood GM, Pinkerton SM, Barlow DH: A prospective randomized single-blind comparative trial of nafarelin acetate with buserelin in long-protocol gonadotrophin-releasing hormone analogue controlled in-vitro fertilization cycles. Hum Reprod 1995;10:293–298
21. Porcu E, Filicori M, Dal PL, Fabbri R, Seracchioli R, Colombi C, Flamigni C: Comparison between depot leuprorelin and daily buserelin in IVF. J Assist Reprod Genet 1995;12:15–19
22. Ron El R, Herman A, Golan A, Van der Venet H, Caspi E, Dietrich K: The comparison of early follicular and mid-luteal administration of long-acting gonadotropin-releasing hormone agonist. Fertil Steril 1990;54:233–237
23. Alvarez C, Cremades N, Blasco N, Bernabeu R: Influence of gonadotrophin-releasing hormone agonist total dose in the ovarian stimulation in the long down-regulation protocol for in-vitro fertilization. Hum Reprod 1997;12:2366–2369
24. Sampaio M, Serra V, Miro F, Calatayud C, Castellvi RM, Pellicer A: Development of ovarian cysts during gonadotrophin-releasing hormone agonists (GnRHa) administration. Hum Reprod 1991;6:194–197
25. Tarlatzis BC, Bili H, Bontis J, Lagos S, Vatev I, Mantalenakis S: Follicle cyst formation after administration of different gonadotrophin-releasing hormone analogues for assisted reproduction. Hum Reprod 1994;9:1983–1986